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Biomarkers

Temporal Relationship and Predictive Value of Urinary Acute Kidney Injury Biomarkers After Pediatric Cardiopulmonary Bypass

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Objectives We investigated the temporal pattern and predictive value (alone and in combination) of 4 urinary biomarkers (neutrophil gelatinase-associated lipocalin [NGAL], interleukin [IL]-18, liver fatty acid-binding protein [L-FABP], and kidney injury molecule [KIM]-1) for cardiac surgery–associated acute kidney injury (AKI).

Background Serum creatinine (S_{Cr}) is a delayed marker for AKI after cardiopulmonary bypass (CPB). Rapidly detectable AKI biomarkers could allow early intervention and improve outcomes.

Methods Data from 220 pediatric patients were analyzed. Urine samples were obtained before and at intervals after CPB initiation. AKI was defined as a ≥50% increase in S_{Cr} from baseline within 48 h after CPB. The temporal pattern of biomarker elevation was established, and biomarker elevations were correlated with AKI severity and clinical outcomes. Biomarker predictive abilities were evaluated by area under the curve (AUC), net reclassification improvement, and integrated discrimination improvement.

Results AKI occurred in 27% of patients. Urine NGAL significantly increased in AKI patients at 2 h after CPB initiation. IL-18 and L-FABP increased at 6 h, and KIM-1 increased at 12 h. Biomarker elevations were correlated with AKI severity and clinical outcomes and improved AKI prediction above a clinical model. At 2 h, addition of NGAL increased the AUC from 0.74 to 0.85 ($p < 0.0001$). At 6 h, NGAL, IL-18, and L-FABP each improved the AUC from 0.72 to 0.91, 0.84, and 0.77, respectively (all $p < 0.05$). The added predictive ability of the biomarkers was supported by net reclassification improvement and integrated discrimination improvement. Biomarker combinations further improved AKI prediction.

Conclusions Urine NGAL, IL-18, L-FABP, and KIM-1 are sequential predictive biomarkers for AKI and are correlated with disease severity and clinical outcomes after pediatric CPB. These biomarkers, particularly in combination, may help establish the timing of injury and allow earlier intervention in AKI. (J Am Coll Cardiol 2011;58:2301–9)

Acute kidney injury (AKI) after cardiac surgery is associated with adverse outcomes, including prolonged intensive care and hospital stays, diminished quality of life, and increased long-term mortality (1). AKI occurs frequently, complicating 30% to 40% of adult and pediatric cardiac surgeries (2,3). Even mild degrees of post-operative AKI portend a significant increase in mortality (4) and morbidity (5). Episodes of AKI may also lead to the development of chronic kidney disease (6).

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AKI diagnosis has relied on a rise in serum creatinine (S_{Cr}) concentration, a delayed and unreliable measure in the acute setting (7). The failure of interventional trials to attenuate AKI after cardiac surgery has been attributed in part to delays in diagnosis. Recent studies have focused on the discovery and validation of early biomarkers of AKI. Initial studies have demonstrated that neutrophil gelatinase-associated lipocalin (NGAL), interleukin (IL)-18, liver fatty

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Abbreviations and Acronyms

AKI = acute kidney injury
AUC = area under the curve
CPB = cardiopulmonary bypass
eCCl = estimated creatinine clearance
ELISA = enzyme-linked immunosorbent assay
IDI = integrated discrimination improvement
IL = interleukin
KIM = kidney injury molecule
L-FABP = liver fatty acid-binding protein
LOS = length of stay
NGAL = neutrophil gelatinase-associated lipocalin
NRI = net reclassification improvement
pRIFLE = pediatric modified Risk, Injury, Failure, Loss, and End-Stage Kidney Disease
RACHS-1 = Risk Adjustment for Congenital Heart Surgery Score version 1
ROC = receiver-operating characteristic
S_{Cr} = serum creatinine

acid-binding protein (L-FABP), and kidney injury molecule (KIM)-1 are individually elevated in the urine early after ischemic AKI (2,8–10). Most studies have focused on the performance of only 1 of these biomarkers to detect AKI before an S_{Cr} rise. The aims of this study were to: 1) evaluate the temporal pattern of elevation in these 4 biomarkers after cardiac surgery; and 2) determine their predictive values, individually and in combination, when added to a clinical predictive model.

Methods

Study design. This study was approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center. All patients <18 years of age undergoing cardiac surgery with cardiopulmonary bypass (CPB) at our center between January 2004 and July 2007 were approached for study inclusion. Patients with severe pre-existing renal insufficiency (S_{Cr} >2 times age-adjusted normal range) were excluded. Written informed consent was obtained before enrollment from the legal guardian of each patient, with assent from the patient when appropriate.

Urine samples for biomarker analysis were obtained immediately before and at 2, 6, 12, and 24 h after initiation of CPB and stored in aliquots at −80°C. The S_{Cr} was routinely measured at baseline (within 72 h before surgery), immediately after surgery, and at least daily in the post-operative period.

The primary outcome was AKI development, defined as a ≥50% increase in S_{Cr} from pre-operative baseline within 48 h of surgery Complexity of surgery was categorized according to the Risk Adjustment for Congenital Heart Surgery Score version 1 (RACHS-1) consensus-based scoring system (11). Secondary outcomes included severity of AKI based on the pediatric modified Risk, Injury, Failure, Loss, and End-Stage Kidney Disease (pRIFLE) criteria (12), duration of AKI, duration of mechanical ventilation, hospital length of stay (LOS), and hospital mortality. We determined pRIFLE by calculation of estimated creatinine clearance (eCCl) using the modified Schwartz formula (13), with "Risk" defined as eCCl decrease of 25% from baseline, "Injury" defined as eCCl decrease of 50%, and "Failure" defined as eCCl decrease of 75% or absolute value <35 ml/min/1.73 m^2. Duration of AKI was defined as the number of days S_{Cr} was ≥50% above baseline. We used dosages of inotropic infusions to calculate an inotrope score at 24 h after CPB to provide a quantified index of the post-operative hemodynamic state (14).

Biomarker measurements. Laboratory investigators were blinded to clinical outcomes. Urine NGAL was assayed using a human-specific commercially available enzyme-linked immunosorbent assay (ELISA, AntibodyShop, Grusbakken, Denmark) (15). Urine IL-18 and L-FABP were measured using commercially available ELISA kits (Medical & Biological Laboratories Co., Nagoya, Japan, and CMIC Co., Tokyo, Japan, respectively) per manufacturer's instructions. The urine KIM-1 ELISA was constructed using commercially available reagents (R & D Systems, Inc., Minneapolis, Minnesota) as described previously (16).

Statistical methods. The analysis subset included patients who had measurement of all 4 biomarkers at baseline and at a minimum of 1 post-operative time point (n = 220) to permit comparisons of biomarkers. Statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, North Carolina) and R version 2.12.1 (R Development Core Team, Vienna, Austria). Demographics, baseline measurements, and clinical outcomes were compared between AKI and non-AKI patients using the nonparametric Wilcoxon rank sum test (continuous variables) or chi-square or Fisher exact tests (categorical variables) as appropriate.

At each time point, the median biomarker measurements were compared between AKI and non-AKI patients and among patients in different pRIFLE strata using the rank transformation approach. Tukey-Kramer adjustment was used to adjust for multiple comparisons at each time point, and adjusted p values were reported. Spearman correlation coefficients were calculated to assess the association between biomarker concentrations at each time point and the clinical parameters.

Univariable logistic regression was used to assess the discriminative ability of biomarkers to predict AKI. Receiver-operator characteristic (ROC) curves were generated for each biomarker at each time point. The areas under the curve (AUC) were compared between biomarkers using the methods developed by DeLong (17). In a secondary analysis, patients with pRIFLE "F" or "I" were assigned as "severe AKI," and we examined the ability of the biomarkers to predict severe AKI.

Multivariable logistic regression analyses were conducted to assess predictors of AKI as well as the performance of the predictive models while combining biomarkers with clinical factors. In addition to biomarkers, potential predictor variables included age, sex, race, CPB duration, prior CPB, RACHS-1 score, and post-operative inotrope score. A parsimonious clinical model (containing clinical factors only) was first determined using backward elimination. The

4 urinary biomarkers were added individually and in combination to the clinical model. Improvements in the model performance were evaluated by AUC, net reclassification improvement (NRI), and integrated discrimination improvement (IDI). No risk categories were selected for the calculation of NRI. R package Hmisc was used for the calculation of NRI and IDI (18,19).

Results

Of the 391 patients enrolled, 220 (56%) were retained for analysis. The 171 excluded patients were among the first 174 patients enrolled. Because their samples were also used for assay optimization, insufficient volume remained for KIM-1 or L-FABP testing. Retained and excluded patients were similar in incidence of AKI (27% vs. 35%, $p = 0.10$) and mortality (0.4% vs. 2.3%, $p = 0.05$). No patient in either group needed dialysis. AKI occurred in 60 of the 220 patients. Retained patient characteristics are shown in Table 1. S_{Cr} increased by a median of 78% in AKI patients. Ninety-eight percent of AKI patients were diagnosed 1 day after surgery. Among AKI patients, 45% developed pRIFLE-R, 35% developed pRIFLE-I, and 20% developed pRIFLE-F.

Patients who developed AKI were younger and had lower baseline S_{Cr}. CPB times were longer in AKI patients, and AKI patients had longer duration of mechanical ventilation and hospital LOS. Baseline urinary biomarker concentrations were similar in AKI and non-AKI groups, with the exception of urine NGAL.

Temporal elevation of biomarkers. As shown in Figure 1, the temporal trends for all 4 urine biomarkers in non-AKI patients were consistently low. In AKI patients, each of the 4 urine biomarkers rose significantly at some time point after CPB initiation. Significant differences between AKI and non-AKI patients were first seen at 2 h for NGAL, at 6 h for IL-18 and L-FABP, and at 12 h for KIM-1 (Figs. 1A to 1D). Once biomarkers were elevated, they each remained elevated in AKI patients at subsequent time points (all $p < 0.0001$).

Figure 2 demonstrates the temporal trend in each urinary biomarker according to pRIFLE. With the exception of

Table 1 Patient Characteristics

Characteristic	No AKI (n = 160)	AKI (n = 60)	p Value
Age, yrs	3.3 (0.5–6.0)	0.6 (0.4–1.8)	<0.0001
Male	84 (53)	26 (43)	0.23
White race	142 (89)	52 (87)	0.67
Prior surgery	76 (48)	24 (40)	0.32
Bypass time, min	92 (67–127)	113 (84–172)	0.003
Baseline S_{Cr}, mg/dl	0.4 (0.4–0.6)	0.3 (0.2–0.4)	<0.0001
Baseline eCCL, ml/min/1.73 dm^2	81.8 (65.0–99.1)	110.6 (84.0–144.6)	<0.0001
Baseline urine NGAL, ng/ml	8.5 (4.0–12.5)	16.0 (10.0–24.0)	<0.0001
Baseline urine IL-18, pg/ml	1.2 (0.0–8.1)	0.9 (0.0–10.0)	0.65
Baseline urine KIM-1, pg/ml	193.9 (83.8–301.4)	211.5 (96.2–377.9)	0.43
Baseline urine L-FABP, ng/ml	7.3 (2.3–27.4)	10.6 (4.2–31.2)	0.34
% S_{Cr} change	0.0 (0.0–25.0)	77.5 (64.6–108.3)	<0.0001
Hospital stay, days	5 (3–7)	7 (5–14)	<0.0001
Ventilator, days	1 (1–2)	2 (1–2)	<0.0001
Inotrope score	0.0 (0.0–0.0)	0.0 (0.0–7.1)	0.19
Death	0 (0)	1 (2)	0.27
RACHS score			0.14
1	20 (13)	4 (7)	
2	68 (43)	29 (48)	
3	60 (38)	24 (40)	
4	9 (6)	0 (0)	
5	2 (1)	1 (2)	
6	1 (1)	2 (3)	
pRIFLE			—
R	—	27 (45)	
I	—	21 (35)	
F	—	12 (20)	
Duration of AKI, days	—	2 (2–3)	—
Dialysis	0 (0)	0 (0)	—

Values are median (interquartile range) for continuous variables (with p values from Wilcoxon rank sum test) or n (%) for categorical variables (with p values from chi-square test or Fisher exact test). pRIFLE and duration of AKI are reported for AKI patients only.

AKI = acute kidney injury; eCCl = estimated creatinine clearance; IL = interleukin; KIM = kidney injury molecule; L-FABP = liver fatty acid-binding protein; NGAL = neutrophil gelatinase-associated lipocalin; pRIFLE = pediatric modified Risk, Injury, Failure, Loss, and End-Stage Kidney Disease; RACHS = risk adjustment for congenital heart surgery score version 1; S_{Cr} = serum creatinine.

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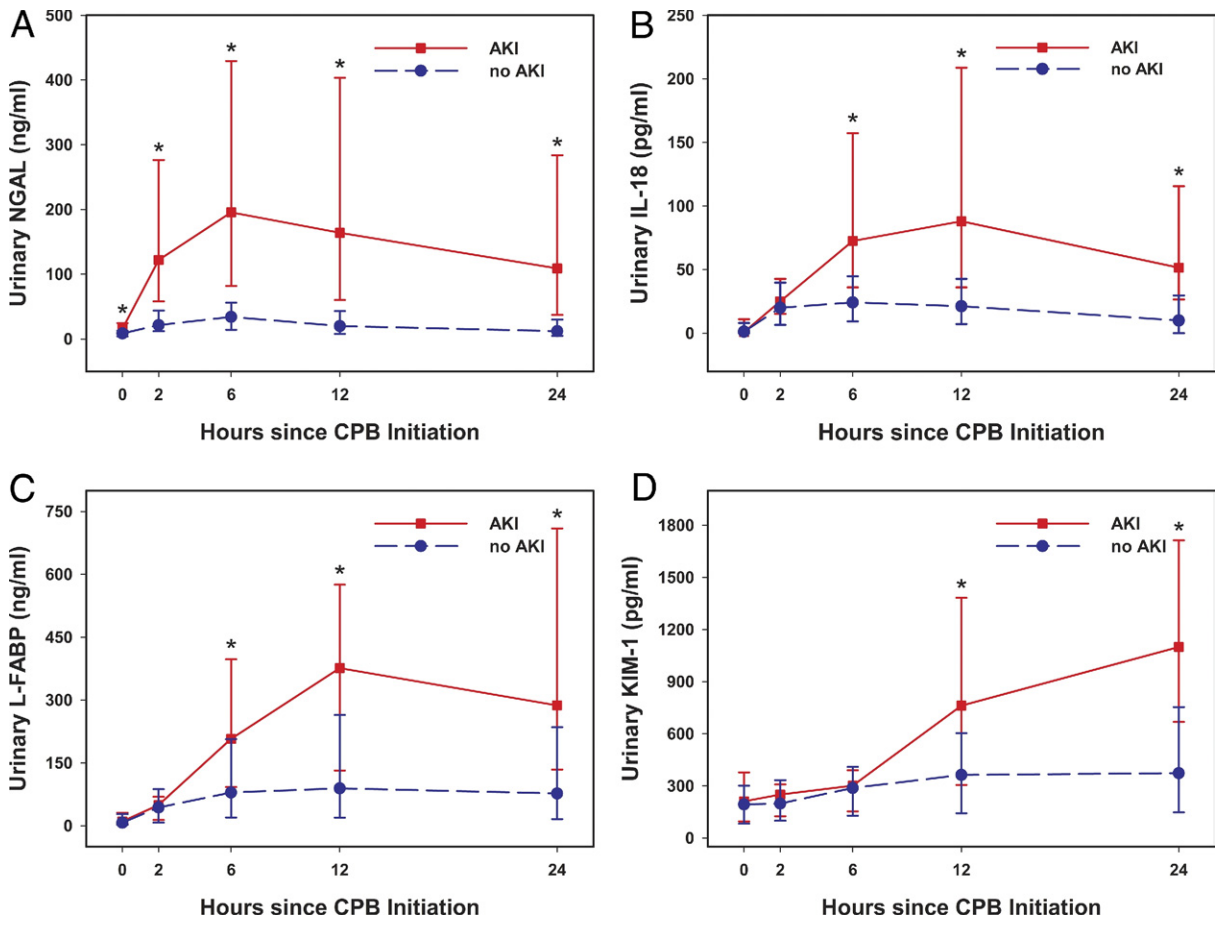

Figure 1 **Urine Biomarker Concentrations by AKI Status**

Median and interquartile range are presented. *Statistically significant differences ($p < 0.0001$) in medians between acute kidney injury (AKI) and non-AKI patients. **(A)** Urine neutrophil gelatinase-associated lipocalin (NGAL); **(B)** urine interleukin (IL)-18; **(C)** urine liver fatty acid-binding protein (L-FABP); **(D)** urine kidney injury molecule (KIM)-1. CPB = cardiopulmonary bypass.

IL-18 in pRIFLE-F patients, a stepwise increase in each biomarker was seen with worsening AKI severity.

Associations between urine biomarkers and clinical characteristics. Spearman correlation coefficients for each biomarker at its earliest elevation (2-h NGAL, 6-h IL-18, 6-h L-FABP, and 12-h KIM-1) with clinical parameters are summarized in Table 2. Urine NGAL at 2 h was correlated with younger age, greater S_{Cr} change, longer CPB time, higher RACHS-1 scores, longer hospital stay, longer ventilation time, and longer duration of AKI. Urine IL-18 at 6 h was correlated with younger age, greater S_{Cr} change, longer hospital stay, and longer ventilation time, but not CPB time, RACH-1 score, or duration of AKI. Urine L-FABP at 6 h and KIM-1 at 12 h were correlated with younger age, greater S_{Cr} change, longer CPB time, higher RACHS-1 scores, longer hospital stay, longer ventilation time, and longer duration of AKI.

To determine whether biomarkers were independently correlated with hospital LOS and ventilator days, we performed Spearman partial correlation, adjusting for the effect of age, CPB time, and RACHS-1 score. At 2 h, NGAL was independently correlated with hospital LOS and ventilator days. At 6 h, L-FABP was independently correlated with hospital LOS, and at 12 h, KIM-1 was independently correlated with ventilator days. IL-18 did not show significant independent correlation with these outcomes.

Predictive ability of biomarkers. Figure 3 demonstrates the progression of ROC curves for each biomarker at each time point, from univariable logistic regression analysis. At 2 h after CPB initiation, urine NGAL was the only predictive biomarker of AKI with an AUC of 0.90, significantly higher than the AUC for any of the other 3 biomarkers (all $p < 0.0001$) (Fig. 3A). At 6 h, urine NGAL, IL-18, and L-FABP were significant predictors of AKI, but NGAL still had the highest discrimination (all $p \leq 0.001$) (Fig. 3B). At 12 h, all 4 biomarkers performed well, with AUC in the good to outstanding range, but urine NGAL exhibited the highest AUC (all $p \leq 0.04$) (Fig. 3C). At 24 h, the predictive value of all 4 biomarkers remained very good, with improvement in the AUC of KIM-1 to

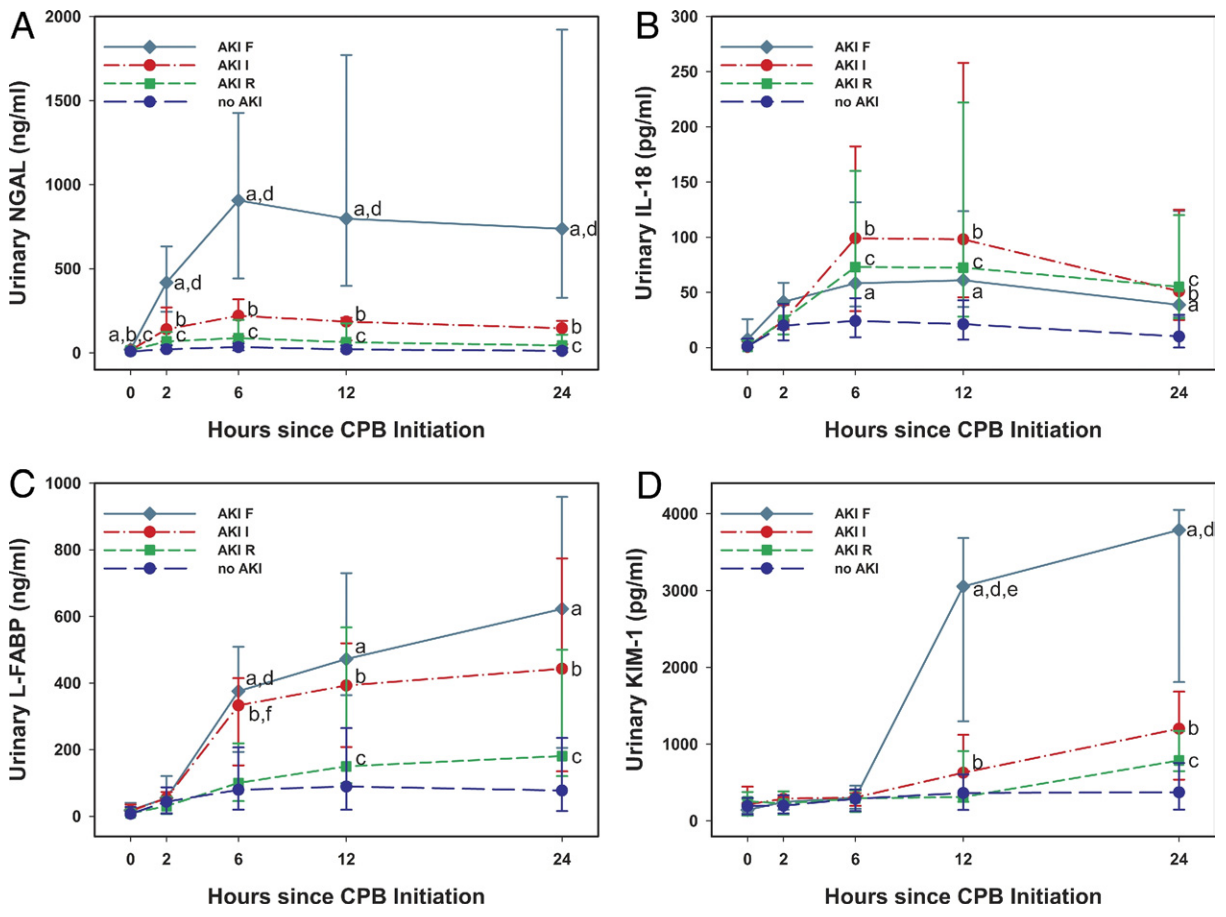

Figure 2 **Urine Biomarker Concentrations by pRIFLE Categories**

Median and interquartile range are presented. Medians were compared between pediatric modified Risk, Injury, Failure, Loss, and End-Stage Kidney Disease (pRIFLE) categories. Statistically significant differences ($p < 0.05$) in medians are denoted by letters: a (F vs. no AKI), b (I vs. no AKI), c (R vs. no AKI), d (F vs. R), e (F vs. I), and f (I vs. R). **(A)** Urine NGAL; **(B)** urine IL-18; **(C)** urine L-FABP; **(D)** urine KIM-1. Abbreviations as in Figure 1.

0.80. At 24 h, NGAL remained significantly better than L-FABP ($p = 0.0004$) but was not significantly superior to either IL-18 ($p = 0.17$) or KIM-1 ($p = 0.11$) (Fig. 3D).

We then performed multivariable logistic regression analyses for the prediction of AKI. Evaluating clinical factors alone, younger age and longer CPB time were independent predictors for AKI. Each biomarker was then added to the clinical model to assess improvement in the predictive ability of the model (Table 3). At 2 h after CPB initiation, adding urine NGAL to the clinical model increased the AUC from 0.74 to 0.85 ($p < 0.0001$). At 6 h, urine NGAL, IL-18, and L-FABP each improved the AUC from 0.72 to 0.91, 0.84, and 0.77, respectively (all $p < 0.05$). At 12 h, all the 4 biomarkers improved the AUC, with urine IL-18 having the most improvement, which was maintained at 24 h after CPB initiation. The added predictive ability of the individual biomarker to the clinical model was also supported by the results of NRI and IDI (Table 3).

Next, in order to assess whether the combination of NGAL with other biomarkers would further improve the predictive ability, we evaluated the performance of the predictive models by adding 1, 2, or 3 other biomarkers (IL-18, L-FABP, KIM-1) to the reference model, including age, CPB time, and NGAL as predictors. At 2 h after

Table 2 **Spearman Correlation Coefficients Between Urinary Biomarker Concentrations and Clinical Characteristics and Outcomes**

	T = 2 h	T = 6 h		T = 12 h
	NGAL	IL-18	L-FABP	KIM-1
Age	−0.21	−0.18	−0.24	−0.29
% S_{Cr} change	0.55	0.34	0.33	0.26
CPB time	0.38	0.11*	0.33	0.14
Hospital LOS	0.44	0.18	0.37	0.28
Ventilator days	0.37	0.22	0.28	0.31
RACHS-1 score	0.24	0.003*	0.21	0.13
Duration of AKI†	0.50	−0.09*	0.31	0.48

*Insignificant correlation coefficients. All other correlation coefficients are significant ($p < 0.05$).
†For AKI patients only.
CPB = cardiopulmonary bypass; LOS = length of stay; T = time; other abbreviations as in Table 1.

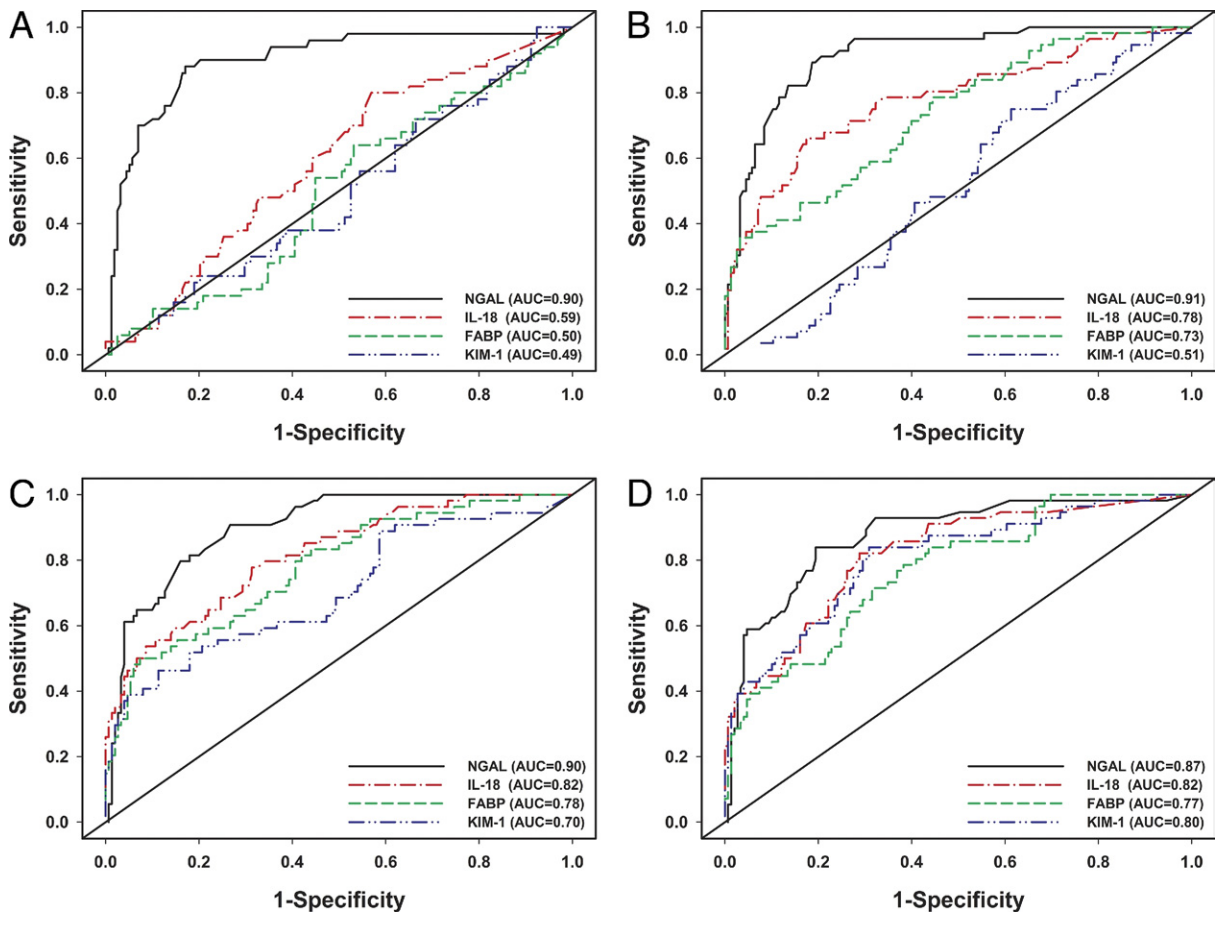

Figure 3 Receiver-Operator Characteristic Curves for Prediction of AKI Using Urine Biomarkers at Time Intervals After CPB

(A) T = 2 h; **(B)** T = 6 h; **(C)** T = 12 h; **(D)** T = 24 h. AUC = area under the curve; T = time; other abbreviations as in Figure 1.

CPB initiation, only urine NGAL predicted AKI, even after other biomarkers were included in the model. At other time points, the significance ($p < 0.05$) of each biomarker in the model, AUC improvement, NRI, and IDI are summarized in Table 4. At the 6-h time point, the combination of NGAL + IL-18 provided the best results in terms of AUC improvement, NRI, and IDI. At 12 h, the best predictive ability was obtained when all 4 biomarkers were included. However, the more parsimonious combination of NGAL + IL-18 + L-FABP provided comparable results. Similarly, at the 24-h mark, the best combination was all 4 biomarkers, but the combination of NGAL + IL-18 + KIM-1 provided comparable results and could be used as the best combination.

Finally, we evaluated the patients with "severe AKI" (pRIFLE-I and -F) to determine the ability of the biomarkers to predict severe AKI. Thirty-three patients were in this group. Our results were very similar to the full AKI cohort

Table 3 Evaluation of the Performance of Predictive Models After Adding a Urinary Biomarker to the Clinical Model

	T = 2 h (n = 208, 50 AKI)			T = 6 h (n = 211, 56 AKI)			TT = 12 h (n = 204, 54 AKI)			TT = 24 h (n = 205, 56 AKI)		
Model	**AUC**	**NRI**	**IDI**	**AUC**	**NRI**	**IDI**	**AUC**	**NRI**	**IDI**	**AUC**	**NRI**	**IDI**
Clinical model	0.74	—	—	0.72	—	—	0.72	—	—	0.72	—	—
+ NGAL	0.85*	0.87†	0.12†	0.91*	1.24†	0.27†	0.85*	1.03†	0.12†	0.82*	0.99†	0.09†
+ IL-18	0.74	0.14	0.007	0.84*	0.93†	0.18†	0.86*	0.86†	0.25†	0.86*	0.84†	0.24†
+ L-FABP	0.73	0.17	0.004	0.77*	0.61†	0.11†	0.81*	0.69†	0.15†	0.79*	0.60†	0.12†
+ KIM-1	0.74	−0.03	0.004	0.73	0.33†	0.006	0.79*	0.44†	0.11†	0.84*	0.83†	0.19†

For NRI, results correspond to % improvement in the performance of the predictive model. For example, NRI = 0.87 means 87% improvement over the reference model (i.e., clinical model: age + CPB time).
*Compared with the clinical model, age + CPB time, statistically significant ($p < 0.05$) improvement in AUC. †$p < 0.05$.
AUC = area under the curve; IDI = integrated discrimination improvement; NRI = net reclassification improvement; other abbreviations as in Table 1.

Table 4 Summary of Biomarker Combinations in the Model: Significance, AUC Improvement, NRI, and IDI

		T = 6 h				T = 12 h				T = 24 h			
Combination	Biomarker	p < 0.05	ΔAUC	NRI	IDI	p < 0.05	ΔAUC	NRI	IDI	p < 0.05	ΔAUC	NRI	IDI
I	NGAL	Yes	0.013	0.64*	0.10*	Yes	0.056*	0.77*	0.21*	Yes	0.069*	0.77*	0.21*
	IL-18	Yes				Yes				Yes			
I	NGAL	Yes	−0.019	0.22	0.02	Yes	−0.002	0.41*	0.09*	Yes	0.005	0.34*	0.08*
	L-FABP	Yes				Yes				Yes			
I	NGAL	Yes	−0.007	0.48*	0.01	Yes	−0.012	0.41*	0.05*	Yes	0.026	0.66*	0.12*
	KIM-1	No				Yes				Yes			
II	NGAL	Yes	0.009	0.60*	0.11*	Yes	0.060*	0.95*	0.27*	Yes	0.073*	0.93*	0.25*
	IL-18	Yes				Yes				Yes			
	L-FABP	No				Yes				Yes			
II	NGAL	Yes	0.013	0.56*	0.10*	Yes	0.058*	0.79*	0.24*	No	0.084*	1.00*	0.27*
	IL-18	Yes				Yes				Yes			
	KIM-1	No				Yes				Yes			
II	NGAL	Yes	−0.003	0.21	0.03	Yes	0.010	0.63*	0.10*	No	0.045	0.84*	0.15*
	L-FABP	Yes				Yes				Yes			
	KIM-1	No				Yes				Yes			
III	NGAL	Yes	0.012	0.61*	0.12*	Yes	0.064*	0.98*	0.28*	No	0.089*	1.01*	0.29*
	IL-18	Yes				Yes				Yes			
	L-FABP	No				Yes				No			
	KIM-1	No				No				Yes			

At 2 h, only NGAL was significant. For NRI, results correspond to % improvement in the performance of the predictive model. For example, NRI = 0.64 means 64% improvement over the reference model (i.e., age + CPB time ± NGAL). *p < 0.05.

ΔAUC = improvement in AUC when compared with the reference model (age + CPB time + NGAL); other abbreviations as in Tables 1 and 3.

(Online Table 1), with similar timing of the biomarker elevations.

Discussion

Our results confirm that urine NGAL, IL-18, L-FABP, and KIM-1 are predictors of post-cardiac surgery AKI. To our knowledge, the current study is the first to: 1) establish a temporal pattern of biomarker elevation after CPB; and 2) demonstrate the utility of biomarker combinations for the improved prediction of AKI beyond clinical models. These findings could enhance the potential for appropriately timed application of therapeutic interventions. Additionally, these biomarkers offer severity and prognostic information at early time points.

The etiology of AKI after CPB is multifactorial and incompletely understood. Because many pathways are involved, it is not surprising that combinations of biomarkers with different properties may prove most predictive. In this study, we found that a clinical model of age and CPB time could predict AKI with reasonable certainty. The addition of biomarkers, however, particularly in combination, significantly increased the predictive value of the model. Thus a panel of strategically selected and rapidly testable biomarkers may prove optimal for early diagnosis and institution of treatment.

We studied 4 biomarkers as candidates for a sequential AKI panel. Urine IL-18, NGAL, L-FABP, and KIM-1 are all up-regulated and released by the kidney tubules during injury and are biological intermediates in the causal mechanisms of ischemia-reperfusion injury to the kidney. These biomarkers are present at low concentrations preoperatively, and their levels increase by several-fold in patients who develop kidney injury. IL-18, a mediator of inflammation, is produced by proximal tubules, and is activated by *caspase-1* after AKI (20). It is more specific to ischemic AKI and is not significantly affected by nephrotoxins or chronic kidney disease. The gene for NGAL is significantly up-regulated in the kidney after ischemic and nephrotoxic injury, and the protein is over-expressed in distal tubule cells (21). NGAL may play a primary role in renal tubular survival and recovery and has been used therapeutically in ischemia-reperfusion injury in animal models (22). Recent studies using an NGAL reporter mouse have established that the kidney is the primary source of urinary NGAL in AKI (23). L-FABP is an inflammatory proximal tubular protein that is up-regulated after a variety of acute kidney injuries (9,24,25). KIM-1 is a transmembrane protein that is over-expressed in de-differentiated proximal tubule cells after ischemic or toxic injury (10,21–23).

We found that urine NGAL concentrations increased at the earliest time point after CPB and were associated with the highest predictive value. We do note that, in some studies, NGAL has not performed as well (26). We hypothesize that this may be related to the confounding disease states (e.g., diabetes, lung disease, inflammation) that may be more prevalent in adults and may affect NGAL concentrations. This further highlights the need for a comprehensive "panel" of biomarkers, optimizing both sensitivity and specificity. Notably, our results demonstrate that combinations of biomarkers provide additional predictive value.

Interestingly, we found small (but significantly) higher pre-operative NGAL levels in patients who developed post-CPB AKI. It is possible that some of our patients had subclinical pre-operative AKI, which may have pre-disposed them to development of AKI.

With the exception of IL-18 in the pRIFLE-F patients, all biomarker concentrations increased in a stepwise fashion with worsening kidney injury. It is unclear why IL-18 did not increase in the same pattern in pRIFLE-F patients, particularly because it has performed well in other studies (8). It is possible that this is due to the small number of RIFLE-F patients analyzed, but further evaluation in larger studies is warranted.

The importance of determining the temporal sequence of the biomarkers is underscored by the fact that the course of experimental AKI proceeds in 4 phases: initiation, extension, maintenance, and recovery (27). The initiation phase is the period during which initial exposure to the ischemic insult occurs, intracellular adenosine triphosphate depletion is profound, and generation of reactive oxygen molecules and labile iron is initiated. Vasodilator, adenosine triphosphate donor, antioxidant, and iron chelation therapies may be especially effective during this phase, and the appearance of the earliest noninvasive biomarkers such as NGAL may be used to trigger such therapies. Prolongation of ischemia followed by reperfusion ushers in the extension phase. Tubules undergo reperfusion-mediated cell death, and the injured endothelial and epithelial cells amplify the inflammatory cascades. This phase probably represents a window of opportunity for early diagnosis with intermediate biomarkers such as L-FABP and IL-18 and active therapeutic intervention with antiapoptotic and anti-inflammatory strategies. During the maintenance phase, both cell injury and regeneration occur simultaneously. Measures such as growth factors and stem cells that accelerate the endogenous regeneration processes, initiated by later biomarkers with high specificity such as KIM-1, may be most effective during this phase.

Study strengths and limitations. Our study has several strengths. First, we used a prospective cohort design and a rigorous protocol to collect specimens, followed by blinded measurements of biomarkers. Second, we enrolled a large, relatively homogenous cohort of subjects in whom the most proximate etiology for AKI would be CPB. The study design also allowed for the precise determination of the temporal rise in biomarkers after CPB.

This study does have important limitations. First, this remains a single-center study, which needs validation at the multicenter level. Second, our results may not be generalizable to adults undergoing CPB or to the myriad other clinical scenarios that commonly lead to AKI in hospitalized patients. Third, S_{Cr} levels were not measured at the same frequency as the urinary biomarkers examined, and the exact timing of S_{Cr} rise in our study population is uncertain. Fourth, the definition of AKI was based on elevations in S_{Cr}, making it very likely that we captured only those with greater than mild injury. Additionally, because patients with congenital heart disease often have decreased muscle mass, elevations in S_{Cr} may reflect more injury than in a healthy population. Indeed, in a recent multicenter pooled analysis of 2,322 critically ill children and adults with cardiorenal syndrome, 20% of patients had early elevations in NGAL but never developed increases in S_{Cr} (28). Importantly, this subgroup of NGAL-positive, S_{Cr}-negative subjects encountered a substantial increase in adverse outcomes, including mortality, dialysis requirement, intensive care unit stay, and prolonged hospital stay. Thus early biomarker measurements may identify patients with subclinical AKI who have an increased risk of adverse outcomes, even in the absence of S_{Cr} rise.

Conclusions

This study is the first to demonstrate the temporal elevation and progression of clinically relevant and predictive biomarkers after AKI and the first to demonstrate the enhanced prediction of AKI using biomarker combinations. The application of biomarker technology to create a bedside AKI "panel" could allow clinicians the ability to pinpoint timing of insult to the kidney and perhaps direct therapeutic interventions. Results of this study support the inclusion of urine NGAL, IL-18, L-FABP, and KIM-1 in such a panel.

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REFERENCES

1. Lok CE, Austin PC, Wang H, Tu JV. Impact of renal insufficiency on short- and long-term outcomes after cardiac surgery. Am Heart J 2004;148:430–8.
2. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231–8.
3. Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J. Independent association between acute renal failure and mortality following cardiac surgery. Am J Med 1998;104:343–8.
4. Lassnigg A, Schmidlin D, Mouhieddine M, Bachmann LM, Druml W, Bauer P, et al. Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. J Am Soc Nephrol 2004;15:1597–605.
5. Zappitelli M, Bernier PL, Saczkowski RS, Tchervenkov CI, Gottesman R, Dancea A, et al. A small post-operative rise in serum creatinine predicts acute kidney injury in children undergoing cardiac surgery. Kidney Int 2009;76:885–92.
6. Goldstein SL, Devarajan P. Acute kidney injury in childhood: should we be worried about progression to CKD? Pediatr Nephrol 2011;26:509–22.
7. Bellomo R, Kellum JA, Ronco C. Defining acute renal failure: physiological principles. Intensive Care Med 2004;30:33–7.

8. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol 2005;16:3046–52.
9. Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P, et al. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 2008;73:465–72.
10. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 2002;62:237–44.
11. Jenkins KJ. Risk adjustment for congenital heart surgery: the RACHS-1 method. Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu 2004;7:180–4.
12. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney Int 2007;71:1028–35.
13. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–37.
14. Wernovsky G, Giglia TM, Jonas RA, Mone SM, Colan SD, Wessel DL. Course in the intensive care unit after 'preparatory' pulmonary artery banding and aortopulmonary shunt placement for transposition of the great arteries with low left ventricular pressure. Circulation 1992;86 Suppl:II133–9.
15. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 2008;3:665–73.
16. Chaturvedi S, Farmer T, Kapke GF. Assay validation for KIM-1: human urinary renal dysfunction biomarker. Int J Biol Sci 2009;5:128–34.
17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
18. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–72, discussion 207–12.
19. Pencina MJ, D'Agostino RB Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med 2011;30:11–21.
20. Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL. Neutrophil-independent mechanisms of caspase-1- and IL-18-mediated ischemic acute tubular necrosis in mice. J Clin Invest 2002;110:1083–91.
21. nMishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534–43.
22. Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24:307–15.
23. Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, et al. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat Med 2011;17:216–22.
24. Ferguson MA, Vaidya VS, Waikar SS, Collings FB, Sunderland KE, Gioules CJ, et al. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. Kidney Int 2010;77:708–14.
25. Negishi K, Noiri E, Doi K, Maeda-Mamiya R, Sugaya T, Portilla D, et al. Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. Am J Pathol 2009;174:1154–9.
26. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009;54:1012–24.
27. Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol 2006;17:1503–20.
28. Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz DN, Wagener G, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury a multicenter pooled analysis of prospective studies. J Am Coll Cardiol 2011;57:1752–61.

Key Words: acute kidney injury ■ biomarkers ■ cardiopulmonary bypass ■ ischemia.

APPENDIX

For a supplementary table, please see the online version of this article.